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Neoplastic Diseases in Small Tropical Fishes[1].

G. M. Smith,
Department of Anatomy, Yale University School of Medicine,
C. W. Coates,
New York Aquarium,
&
L. C. Strong,
Department of Anatomy, Yale University School of Medicine.

(Plates I-III).

Although many thousands of the smaller tropical fishes have been under observation at the New York Aquarium during the past five years, representing approximately 400 species, it is of interest to note that in only five species has a tendency toward neoplasia been observed to date. These species were the following:

1. Hybrids of the Mexican killifish, *Xiphophorus helleri* Heckel and *Platypoecilus maculatus* Guenther.
2. *Rasbora lateristriata* (Bleeker).
3. *Rasbora trilineata* Steindacher.
4. *Rasbora daniconius* (Hamilton-Buchanan).
5. *Heterandria formosa* Agassiz.

Before 1875 tumors in fishes were practically unknown. It was believed by many early pathologists that tumors were a characteristic of man and the warm blooded animals in general, and that such new growths did not occur in cold blooded creatures. It was Bugnion (1875) who is usually credited with the earliest precise description of a fish tumor. This growth was a giant cell sarcoma and occurred in the small European fish called the Ellritze—*Phoxinus laevis*. With the end of the last century and the beginning of the new, greater interest manifested itself in the study of tumors in fishes, due largely to the widespread effort to learn about the origin of cancer. As the result of the careful studies of Plehn (1906), Johnstone (1915), Takahashi (1929), Thomas (1931), Haddow and Blake (1933) and other investigators, various types of tumors in many species of fishes were described. But even today our knowledge of fish tumors remains very incomplete. There is little information regarding pathogenesis and course of these hyperplastic lesions. We have no idea about the factors which, on the one hand favor the growth of these tumors and, on the other, inhibit their progress. One difficulty has been that relatively few tumors come under observation, as it is well known that fishes succumb rapidly to any form of disease and sick fishes are attacked and destroyed by other fishes.

Some peculiarities of fish tumors are now recognized. For example,

[1] From the Department of Anatomy, Section Neuro-anatomy, Yale University School of Medicine, and the Laboratory of the New York Aquarium.

they remain very much localized and seem to be less infiltrative and destructive than mammalian and avian tumors. Transplantation of tumor tissue from fish to fish, though frequently attempted, has been uniformly unsuccessful, even when this has been undertaken with great care and exacting technique. The occurrence of secondary growths or metatases, so frequently seen in mammals and in birds, is rarely encountered in the case of fishes. This has been thought to be due to the fact that the lymphatic system of the fish is merely a diffuse arrangement of capillaries and spaces possessing no organized lymphatic glands (Haddow and Blake, 1933). Secondary tumors in fishes seem to be the exception even with such growths as the black pigmented melanoma, which in the human being is most malignant and causes many widely scattered secondary growths followed by rapid death. Owing to the numerous cutaneous pigment cells, containing granules of different colors (black, red, yellow, etc.), the skin of fishes has proved unique for the study of pigmented skin tumors. Some of these show a marked difference from the skin tumors arising in terrestrial animals. Thus, beside the commoner grayish-white fibromas of the skin, there are known to be greenish-yellow tumors of the skin, red tumors or erythrophoromas, black tumors or melanomas, and even a silvery irridescent tumor composed of cells containing irridescent crystals. The last tumor has been described by the Japanese investigator Takahashi (1929), and he has named this a guanophoroma. All these colored cutaneous tumors in fishes are highly prized at the present time, because some of them occur in the extensively inbred small tropical fishes. They are furnishing an accurate means of studying certain genetic principles underlying the causes of tumor growth and cancer.

A neoplastic disease designated by Reed and Gordon (1931) as "Melanotic Over-growth," is shown in Plate I, Fig. 1. It occurs in the hybrids of the small Mexican killifish. Several instances of this condition have come under our own observation. The disease has been studied extensively from the genetic standpoint by the European investigators Haüssler (1928) and Kosswig (1929) as well as by the Americans Reed and Gordon (1931). The condition is characterized by a great multiplication of the black pigment cells or melanophores which normally lie in small collections in the derma directly under the transparent epidermis. With massing of these newly formed melanophores, the affected part of the skin turns an intense black. Very large collections of melanophores may actually form tumors which penetrate into deeper lying muscles and destroy adjacent tissues. Thus, in the later stages of the disease, the tail and the tissues at the base of the tail may become invaded, destroyed and lost by a process of sloughing. (Pl. I, Fig. 2).

In another of the hybrids of *Platypoecilus maculatus* and *Xiphophorus helleri*, which is highly pigmented in red, there arose a brick red tumor in the region of the dorsal fin. (Pl. I, Fig. 3). This small mass had its origin in a field of red pigmented cells (erythrophores) seen as an irregular red patch in the mid dorsal region. The tumor invaded the skin and the tissues of the dorsal fin, but not the deeper lying muscles. Histologically, the cells composing the tumor are rather large, round, oval, or somewhat fusiform (Pl. II, Fig. 5). They show at times small protruding branches or dendrites. The nucleus of the cell is small, and usually centrally located. The body of the cells, prepared by the method of frozen sections, contained minute granules of a dark red or orange color. These granules assumed a bright red stain in the presence of Scharlach R., and were regarded as lipoidal in character. In sections prepared by the paraffin method, the pigment granules become dissolved, and the cytoplasm of the cells appear shrunken and vacuolated. The red or orange pigment granules give the characteristic red tint to the tumor during life. No black pigmented cells were noted in any of the sections examined. The supporting tissue of the tumor cells

are very delicate connective tissue cells which support a fine network of small capillaries. Nerves were not noted. The overlying epithelium of the skin consists of several layers of cells, but without any appreciable thickening.

Erythrophoromas in fishes are distinctly rare. Thomas (1931) of France described three such tumors in relatively large fishes. One occurred in a tunny fish, and the other two were in the trout. More recently a metastasizing cutaneous erythrophoroma was noted in a winter flounder caught in the waters of Long Island Sound (Smith, 1934). Two instances of erythrophoroma were produced experimentally by Kosswig (1929) by cross breedings between *Platypoecilus maculatus* and *Xiphophorus helleri.*

Discussion on the Genetics of Fish Tumors. (L. C. Strong).

Susceptibility to spontaneous tumor formation has been very completely demonstrated, on a genetic basis, by Reed and Gordon (1931) in hybrids of the Mexican killifish. They showed, in the first place, that the production of macromelanophores (the histological unit involved in the formation of the larger black areas of the fish) is inherited as a sex-linked dominant factor; secondly, that another factor, an intensifier, found in *Xiphophorus helleri,* is also inherited as a dominant; thirdly, that both factors in their original location manifest themselves in an orderly manner; lastly, that if both determiners are introduced into a hybrid fish, "macromelanophore invasion results in a state of general melanosis in which there is a deterioration and final complete replacement of normal tissues by the invading cells. A state is reached where there occur sharply delineated overgrowths, the final tissue of the affected part is clearly a neoplasm." Thus it was determined that hybridization, used for the production of new varieties by recombinations of variations in diverse species, had actually given a combination of genetic determiners that led invariably to tumor formation. The very important question arises whether these same factors in the original species, not influenced by other determiners from other species, could, by themselves, produce an abnormal growth such as a tumor. The problem presents itself to a geneticist, "Has something gone wrong with the genetic factor or factors which control the presence and physiological activity of the erythrophore (red pigmented cell) in this fish where an erythrophoroma was produced?" The question is an important one not only from the standpoint of the fish fancier but also to the student of neoplasms in general. The problem needs further investigation. It could be worked out just as completely as Reed and Gordon have done with the black tumors by the following procedure: 1. The histological examination of fishes with red tumors received by a pathologist when the fishes are still alive, and (2) the statistical analysis of the descendants of a fish known to have a red tumor at some time during its life. It is hoped that fish fanciers may become interested in the presence of tumors in their stocks and that they will make available to other investigators such material as may come to their attention.

Contrasting with these neoplasms in hybrids of the Mexican killifish above discussed was a small solitary fibrous growth seen in *Rasbora daniconius* (Hamilton-Buchanan), (Pl. I, Fig. 4). This was a spontaneous tumor in the region of the dorsal fin, sharply circumscribed, black in color externally where the melanophores of the derma have aided in forming a capsule around it. On sectioning the tumor, however, it was noticed that the black color was strictly limited to the periphery of the growth, and that the interior was composed of firm white fibrous tissue. Microscopically

the tumor is composed of loosely arranged interlacing bundles of elongated connective tissue cells, in places hyaline in character (Pl. II, Fig. 6). The blood vessels are very few in number. The tumor appeared to belong to the group of fibromas and was regarded as benign in nature. Melanophores were irregularly scattered over the surface of the tumor, and the overlying epidermis was destroyed. The melanophores were doubtless corial in their origin and did not constitute a part of the tumor as such.

Epithelial and glandular tumors in this small series of neoplasms were represented by two hyperplastic growths of the thyroid gland, the first occurring in *Rasbora lateristriata* (Pl. III, Fig. 9) and the second in *Heterandria formosa* Agassiz, (Pl. III, Fig. 10). Microscopically, these tumors resemble each other fairly closely in that they are composed of masses of thyroid tissue, partly in a dense compact arrangement of cells without acini or follicles, partly in closely grouped follicles distended with varying amounts of colloid material (Pl. II, Fig. 7). Both tumors in the extension of their growth have encroached upon the structures of the gills, so that the thyroid tissue replacing in part the epithelium of the gills lies in closest contact with bone, cartilage, and muscle tissue. There is no tendency to form a capsule.

To what extent the invasion of the gill structures can be regarded as an evidence of malignancy in the case of thyroid tumors in fishes, is still a matter of individual interpretation. It is not unlikely, however, that such an extension beyond normal topographical limits on the part of a massed growth of thyroid tissue encroaching upon bone, cartilage, muscle and epithelium, may actually represent neoplasia of the thyroid gland of a varying degree of malignancy. In both our fishes, the growth regarded as adenocarcinoma, was a spontaneous one, forming an appreciable sized tumor without metastases. Other fishes in these same tanks were not affected in any way, strengthening the belief that the disease was not infectious. Similar tumors occurring in other fishes, notably in the trout, have been designated as adenocarcinoma. Widely studied in Europe and in this country by Gaylord and Marsh (1914), Marine and Lehnhart (1910), the disease has been attributed to an unknown agent in the water, perhaps of an infectious nature, causing a disturbance in nutrition or metabolism. The disease has not been transmitted by transplanting the diseased thyroid tissue into other fishes of the same order.

The last tumor of the present series of tumors in small tropical fishes occurred in *Rasbora lateristriata* (Bleeker). This fish developed a swelling in the upper abdominal region near the liver. The intra-abdominal swelling could be seen distinctly during life through the semitransparent abdominal wall of the fish. After approximately two months of observation, the swelling extended cephalically, and presently it was noticed that the right operculum was pressed outward by a mass of whitish tissue involving the gill on the right side (Pl. III, Fig. 11). The health of the fish became impaired and it was then sacrificed for the purpose of histological study. Serial microscopic sections were prepared of the entire head and the abdomen.

The tumor tissue is composed of closely packed small lymphoid cells, with deeply staining nuclei and scant cytoplasm. There are many small necrotic areas where the determination of the structure of the tissue is difficult. Here and there, intermingling with the masses of small lymphoid cells, are larger paler vacuolated cells with vesicular nuclei, which suggest structurally a larger type of lymphoid cell or a degenerating form of lymphoid cell. The exact site of origin of the tumor cannot be determined by the microscopic study of the sections. The principal mass lies in the abdomen ventral to the liver, spleen, kidney, gastro-intestinal tract and ovarian tissue, yet in closest relation with all these organs. Plate II, Fig. 8

shows the tumor close to a part of the stomach. There is a distinct fusion between tumor and the ventral peritoneum at one point, suggesting that the neoplasm had its beginning in lymphatic tissue near the peritoneum. The tumor was regarded as a lymphosarcoma with invasive malignant properties. It extended as an irregular mass from the abdomen in a cephalic direction, passing to the right of the heart to the roof of the oral cavity, reaching outward and to the right to involve and destroy in large part the tissues of the gill. There was also an extension of the tumor into the cranial cavity, where it reached as far as the base of the brain and laterally to the lower boundaries of the right and left saccule of the auditory apparatus. The tumor encroached upon muscle, bone, and cartilage wherever these structures were encountered in its course, causing in places their destruction.

We found no parasites in tumor tissue, although several small encysted parasitic larvae were noted in the abdomen at points remote from the growth. The examination of the blood found in cross sections of the heart and the larger blood vessels indicated no increase in the white cells, such as might be expected in a leukaemic state if this were present.

Summary.

In this paper several neoplastic diseases are described occurring in certain species of the small tropical fishes. These are (1) "Melanotic Over-growths" and a red pigmented tumor (erythrophoroma) in hybrids of *Xiphophorus helleri* Heckel and *Platypoecilus maculatus* Guenther; (2) Fibroma in *Rasbora daniconius* (Hamilton-Buchanan); (3) Adenocarcinoma of the thyroid in *Rasbora trilineata* Steindacher and *Heterandria formosa* Agassiz; (4) an extensive lymphosarcoma in *Rasbora lateristriata* (Bleeker). The first group of pigmented cutaneous tumors in the hybrids of the Mexican killifish are discussed from the genetic viewpoint.

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EXPLANATION OF THE PLATES.

Plate I.

Figs. 1 and 2. Melanotic overgrowth occurring in hybrids of the Mexican killifish. Fig. 1 shows a moderately severe black pigmentation of parts of the skin, while Fig. 2 shows an advanced lesion with a tumor-like mass near the base of the tail which has been almost completely destroyed with the progress of the disease.

Fig. 3. A red pigmented tumor (erythrophoroma) growing in a hybrid of the Mexican killifish *Platypoecilus maculatus* and *Xiphophorus helleri*. The growth involves the region of the dorsal fin. Drawing made from living fish.

Fig. 4. *Rasbora daniconius*, with a small fibroma growing on the back of the fish near the dorsal fin. The fibroma is surrounded with a capsule containing black pigment cells. Drawing made after death of the fish.

Plate II.

Fig. 5. Photomicrograph of erythrophoroma occurring in fish represented in Plate I, Fig. 3. Large, round, oval or fusiform cells with relatively small nucleus. These cells in the fresh condition contained granules of red or orange pigment, which gave the red tint to the tumor. x 230.

Fig. 6. Photomicrograph of tumor occurring in *Rasbora daniconius*, depicted in Plate I, Fig. 4. The tumor is composed of interlacing bundles of elongated connective tissue cells. A moderate number of black pigmented cells, called melanophores M, are shown in the periphery of the growth. x 80.

Fig. 7. Photomicrograph of thyroid tumor occurring in fish depicted in the photograph Fig. 9. A, compact thyroid growth; B, an area of the growth showing thyroid tissue arranged in follicles; C, thyroid tissue infiltrating the structure of the gills, G. x 85.

Fig. 8. Photomicrograph of lymphosarcoma X in relation to area of the stomach, S. x 200.

Plate III.

Fig. 9. *Rasbora lateristriata* showing neoplasm of the thyroid.

Fig. 10. *Heterandria formosa* with neoplasm of the thyroid.

Fig. 11. Photograph of the ventral side, *Rasbora lateristriata*. A, indicates mass occupying the abdomen, and B, the extension of the growth to the gill of the right side.